ACTIVE CARBONATES OF POLYALKYLENE OXIDES FOR MODIFICATION OF POLYPEPTIDES

This is a continuation-in-part of U.S. Ser. No. 340,928, filed Apr. 19, 1989, now abandoned, the contents of which are hereby incorporated by reference into the present application.

BACKGROUND OF THE INVENTION

The present invention relates to chemical modification of polypeptides by means of covalent attachment of strands of polyalkylene oxide to a polypeptide molecule such as is disclosed in U.S. Pat. No. 4,179,337, to Davis, et al. It is disclosed in Abuchowski & Davis "Enzymes as Drugs", Holcenberg & Roberts, eds., pp. 367–383, John Wiley & Sons, N.Y. (1981) that such preparations of polypeptides have reduced immunogenicity and antigenicity and also have a longer lifetime in the bloodstream as compared to the parent polypeptides. These beneficial properties of the modified polypeptides make them very useful in a variety of therapeutic applications, such as enzyme therapy.

The active groups that are introduced onto polyalkylene oxides for the purpose of subsequent attachment of these polymers to proteins must satisfy the following requirements:

1. The active groups have to be reactive enough to afford fast reaction with a protein under mild conditions;
2. The residues released from the active groups during the process of modification have to be nontoxic and/or readily separable from the protein-polymer adduct.

To effect covalent attachment of polyethylene glycol (PEG) to a protein, the hydroxyl end-groups of the polymer must first be converted into reactive functional groups. This process is frequently referred to as "activation" and the product is called "activated PEG". Methoxypolyethylene glycol (mPEG) derivatives, capped on one end with a functional group, reactive towards amines on a protein molecule, are used in most cases.

The most common form of activated PEG heretofore used for preparation of therapeutic enzymes is poly-(ethylene glycol) succinoyl-N-hydroxysuccinimide ester (SS-PEG) [Abuchowski, et al. Cancer Biochem. Biophys. 7, 175–186 (1984), Scheme 1]. Use of this activated polymer satisfies both of the requirements listed above. However, it has one major drawback. The ester linkage between the polymer and succinic acid residue has limited stability in aqueous media [U.S. Pat. No. 4,670,417, to Iwasaki, et al. (1987); Ulbrich, et al., Makromol. Chem. 187, 1131–1144 (1986)]

Scheme 1:

Conventional attachment of PEG to a protein using SS-PEG as the activated form of the polymer

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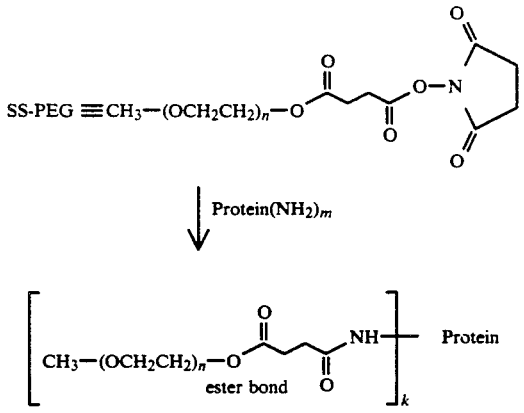

Various functionalized polyethylene glycols (PEG) have been effectively used in such fields as protein modification (Abuchowski & Davis, 1981, supra), peptide chemistry [Zalipsky, et al., Int, J. Peptide Protein Res., 30, 740–783 (1987)] and preparation of conjugates with biologically active materials [Zalipsky, et al., Eur. Polym. J. 19, 1177–1183 (1983) and Zalipsky and Barany, Polymer Preprints, Am. Chem. Soc. Div. Polym. Chem. 27(1), 1–2 (1986)]. PEG protein conjugates useful in medical applications have shown promise, particularly with regard to their stability to proteolytic digestion, reduced immunological response and longer half-life times in the bloodstream.

To accomplish this, the prior art has activated the hydroxy group of PEG with cyanuric chloride and the resulting compound then coupled with proteins (Abuchowski, et al. (1977) J. Biol. Chem. 252, 3578; Abuchowski and Davis, 1981, supra). However, various disadvantages of using this method exist, such as the toxicity of cyanuric chloride and the non-specific reactivity for proteins having functional groups other than amines, such as free essential cysteine or tyrosine residues.

In order to overcome these and other disadvantages, alternative procedures, such as succinimidyl succinate derivatives of PEG (SS-PEG) have been introduced (Abuchowski, et al. 1984, supra, see Scheme 1, above). It reacts quickly with proteins (30 min) under mild conditions yielding active yet extensively modified conjugates use of this activated polymer has one major disadvantage. The ester linkage between the polymer and the succinic acid residue has limited stability in aqueous media [U.S. Pat. No. 4,670,417 to Iwasaki, et al. and Ulbrich, et al. Makromol Chem., 187, 1131–1144 (1986)].

Formation of urethane linkages between amino groups of a protein and PEG overcomes the problem of hydrolytic loss of the polymer chains [Veronese, et al., Appl. Biochem. Biotechnol. 11, 141–152 (1985)]. In fact, it was demonstrated on radioactively labeled PEG-derivatives that urethane links are completely stable under a variety of physiological conditions [Larwood & Szoka J., Labeled Compounds Radiopharm., 21, 603–614 (1984)]. The attachment of PEG to a protein via a carbamate derivative was accomplished [Beauchamp, et al. Analyt. Biochem. 131, 25–33 (1983)] using carbonyldiimidazole-activated PEG. However, the polymer activated in this manner is not very reactive and therefore long reaction times (48–72 hrs at pH 8.5) were